Journal of Medicinal Chemistry

ARTICLE

pubs.acs.org/jmc

A One-Pot Synthesis and Biological Activity of Ageladine A and Analogues

Sudhir R. Shengule,[†] Wendy L. Loa-Kum-Cheung,[†] Christopher R. Parish,[‡] Mélina Blairvacq,[§] Laurent Meijer,[§] Yoichi Nakao,[∥] and Peter Karuso*[,†]

[†]Department of Chemistry & Biomolecular Sciences, Macquarie University, Sydney NSW-2109, Australia

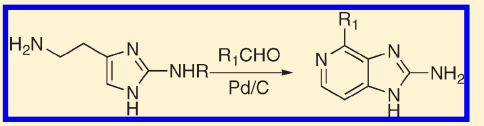
[‡]Division of Immunology and Genetics, John Curtin School of Medical Research, Australian National University, Canberra ACT 2601, Australia

[§]CNRS, Protein Phosphorylation and Human Disease Group, Station Biologique, 29682 Roscoff cedex, Bretagne, France

[∥]School of Advanced Science and Engineering, Waseda University, 3-4-1, Okubo, Shinjuku-ku, Tokyo, 169-8555, Japan

Supporting Information

ABSTRACT: A one-pot synthesis of ageladine A and analogues is reported. The key Pictet–Spengler reaction between 2-aminohistamine and aryl aldehydes has been successfully utilized for the synthesis of the natural product and 14 analogues. These compounds were screened for their matrix metalloprotease (MMP) and kinase inhibition to develop the first structure–activity relationship of ageladine A analogues. One compound, which showed significant kinase activity but little MMP inhibitory activity, was found to be highly active in an antiangiogenic screen, suggesting that the angiogenic activity of ageladine A is not associated with MMP inhibition but rather kinase inhibitory activity. Cytotoxicity was excluded as a mode of action by the assay of ageladine A and an analogue against 60 human cell lines.



INTRODUCTION

Ageladine A (**1**), an anticancer natural product, was recently isolated from the marine sponge *Agelas nakamurai* by Fusetani and co-workers.[1] The compound is the first example of an imidazolopyridine natural product that formally belongs to the oroidin family of marine natural products (pyrrole-imidazole alkaloids). **1** has shown inhibition against various matrix metalloproteinases (MMPs) at micromolar levels and strong antiangiogenic activity that is believed to be associated with its MMP inhibition.[2] However, **1** does not function as an MMP inhibitor through zinc complexation and thus has a novel mode of action.[1] The important biological activity and unusual compact structure have made **1** an important target for the synthetic chemist. To date, four total syntheses have been reported: Weinreb reported the first total synthesis of **1** using a 6π-azaelectrocyclization and Suzuki–Miyaura coupling of *N*-Boc-pyrrole-2-boronic acid and chloropyridine as a key step.[3,4] Soon after, we reported the concise synthesis of **1** based on biomimetic principles.[5] The biogenesis of **1** is likely to be from proline and histidine, which could produce 4,5-dibromo-2-formylpyrrole and 2-aminohistamine (2-AH). Retrosynthetic analysis suggested that an imine formed between 2-AH and 2-formylpyrrole could cyclize via a Pictet–Spengler reaction to yield the requisite skeleton of the **1** in one step. Utilizing this approach, we were able to synthesize **1** in two steps from 2-AH and *N*-Boc-4,5-dibromo-2-formyl pyrrole (Scheme 1).[6] Weinreb reported the third total synthesis using a variation of his original method to overcome the low yield of the last step but still employing a 6π-2-azatriene electrocyclization for the formation of the imidazolopyridine moiety.[4,7] Most recently, Ando et al. reported a fourth synthesis of **1**, also based on the Pictet–Spengler cyclization between the *N*-Boc-2-AH and *N*-protected 2-formyl pyrroles.[8] In this case, the dehydrogenation was effected in two steps using IBX and then MnO_2 instead of choranil, which was found to improve the overall yield. Herein we report the one-pot synthesis of **1** and a range of analogues from 2-AH and various heterocyclic aldehydes. The analogues synthesized were further screened for their MMP-2, MT1-MMP, and/or kinase inhibition. A small number were further screened for cytoxicity and their ability to inhibit angiogenesis. Surprisingly, the 2-pyridine analogue, which displayed poor MMP activity, was more active than the natural product in the angiogenesis assay.

RESULTS

The Pictet–Spengler reaction between the 2-AH and an aldehyde was utilized for the synthesis of a range of tetrahydroageladine A analogues (Table 1). While the reaction is accelerated by the presence of a Lewis acid such as $Sc(OTf)_3$,[6] it also proceeds under base catalysis (triethylamine, potassium carbonate) and with no catalyst, albeit at a slower rate. Utilizing 1.5 equiv of triethylamine in ethanol was found to be the most robust method for condensing various heterocyclic aldehydes with 2-AH or *N*-boc-2-AH. Dehydrogenation was achieved in reasonable to good yield using chloranil in chloroform for all the

Received: January 13, 2011
Published: March 17, 2011

Scheme 1. Biomimetic Synthesis of Ageladine A

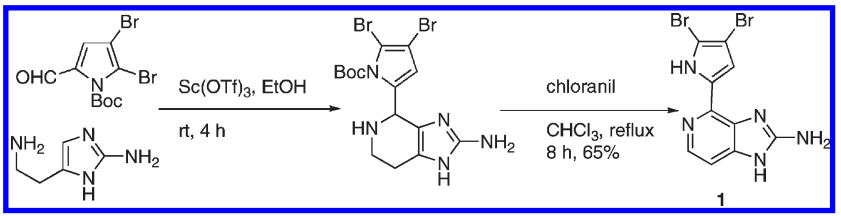
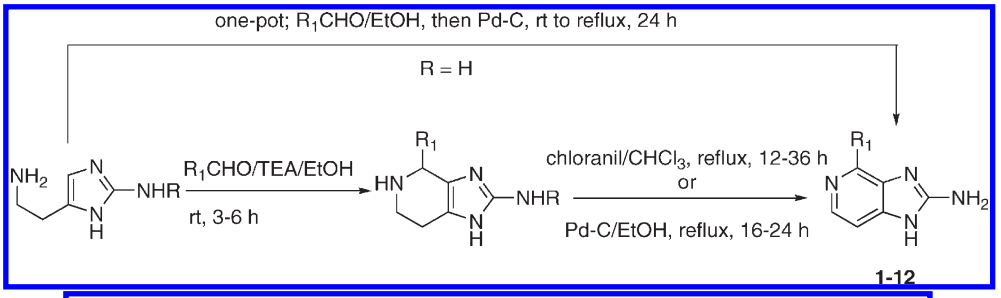
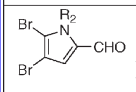
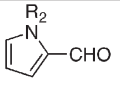
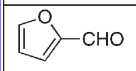
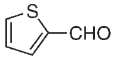
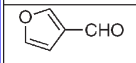
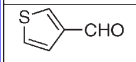
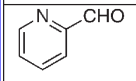
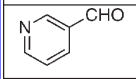
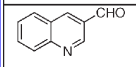
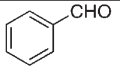
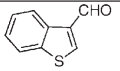
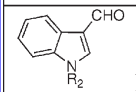


Table 1. Synthesis of Ageladine A and Analogues



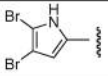
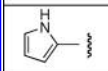
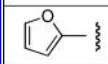
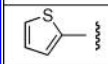
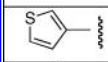
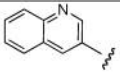
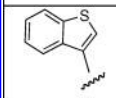
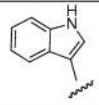
R_1CHO	R	Tetrahydrointermediate structure and yield (%)[a]		Final product structure and yield (%)	
 R_2=Boc	H	R_2=Boc	44[b]	**1** R_2=H	65[b], 16[d]
 R_2=Boc	H	R_2=Boc	65	**2** R_2=H	54[b], 33[d]
	Boc		92	**3**	62[b], 73[d]
	Boc		74	**4**	70[b], 87[d]
	Boc		–	**5**	–, 76[d]
	Boc		80	**6**	80[b], 86[d]
	H		89	**7**	87[c], 81[d]
	H		79	**8**	80[c], 75[d]
	H		82	**9**	74[c], 70[d]
	H		–	**10**	–, 59[d]
	Boc		70	**11**	64[b], 76[d]
 R_2=Boc	H	R_2=Boc	56	**12** R_2=H	71[b], 36[d]

[a] R_1CHO, TEA, EtOH, rt, 3−6 h. [b] Chloranil, $CHCl_3$, reflux, 12−36 h. [c] Pd/C, EtOH, reflux, 16−24 h. [d] One-pot method; R_1CHO, EtOH, then Pd/C, rt to reflux, 24 h.

analogues except those containing a pyridine (or quinoline), where the yields were lower. The use of palladium on charcoal for dehydrogenation gave excellent yields for the pyridine and quinoline analogues.

dx.doi.org/10.1021/jm200039m |J. Med. Chem. 2011, 54, 2492–2503

Table 2. MMP Inhibition (IC_{50}) Results for Ageladine A and Analogues

R=	compound	MMP-2 (μg/mL)	MT1-MMP (μg/mL)
	**1**	1.7	0.2
	**2**	10	1.6
	**3**	12.4	3.2
	**4**	3.0	0.57
	**6**	19	6.4
	**7**	22%[a]	6%[a]
	**8**	5%[a]	–
	**9**	11%[a]	9%[a]
	**11**	21%[a]	17%[a]
	**12**	31%[a]	2.8

[a] Percentage inhibition at 20 μg/mL.

A one-pot procedure, entailing stirring 2-AH and an aldehyde in ethanol for 6 h, after which Pd/C was added and the mixture refluxed for 24 h. This gave good yields of all the ageladine A analogues except for pyrrole aldehydes, where the two-step procedure gave better yields (Table 1, last column)

Biological Screening of Ageladine A Analogues. *Matrix Metalloprotease Inhibition.* Compound **1** shows inhibitory activity against MMP-1, -2, -8, -9, -12, and -13 with the IC_{50} values of 2.0, 1.2, 0.39, 0.79, 0.33, and 0.47 μg/mL, respectively.[9]

Eleven analogues (**2**−**12**) were screened for their potential inhibition of MMP-2 and MT1-MMP (MMP-14) (Table 2). Compounds where the pyrrole ring is replaced by other five-membered rings showed comparable or slightly reduced activity. For example, the thiophene analogue (**4**) showed activity similar to **1**. When the sulfur atom is replaced by oxygen (**3**), however, there is almost an order of magnitude decrease in activity. The thiophene regioisomers (**4** and **6**) also showed that much better activity is obtained if the heteroatom is in the 2-position. The analogues where the five-membered ring is replaced with pyridine (**7**, **8**), benzothiophene (**11**), and quinoline (**9**) showed virtually no activity, indicating that a small ring size may be important for activity. Interestingly, the indole derivative (**12**) did show selective activity against MT1-MMP, indicating that a larger ring size might be accommodated by some MMP enzymes. However, none of the analogues showed better activity against MMP's than the natural product.

Angiogenesis Inhibition. Previous studies have shown that **1** (10 μg/mL) inhibits angiogenesis from vascular progenitor cells in 3D culture using a type-I collagen gel, but its activity was not quantified nor compared to any known antiangiogenic drug. Table 3 and Table S1 (Supporting Information) show the comparative data for ageladine A and eight analogues with positive and negative controls using the rat aorta outgrowth in vitro assay.[10]

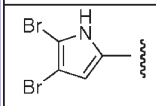
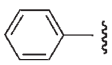
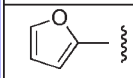
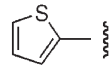
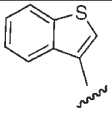
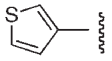
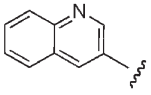
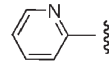
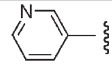
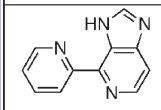
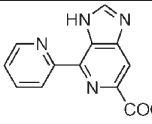
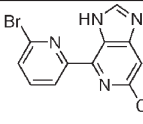
The most potent analogue in the angiogenesis assays was **7**, which is 14× more active than PI-88 (phosphomannopentaose sulfate; **13**),[11] the positive control, and 3× more active than ageladine A in the angiogenesis assay. Several related compounds were synthesized from histidine and histamine and 2-formylpyridine (**14**−**16**), which showed weaker activity but still better than **13**. Adding a lipophilic bromine onto the pyridine ring (**16**) reduced activity but a carboxylate on the lower pyridine (**15**) seems to be tolerated.

Cytotoxicity Screening. Ageladine A and analogue **7** were tested by the NCI against a panel of 60 human cancer cell lines for anticancer activity (Figure S2, Supporting Information).[12] Ageladine A was found to be nontoxic at 10 μM and was not tested further. Compound **7** inhibited the growth of one cell line (renal cancer CAKI-1) by 95% and stimulated the growth of one cell line (Melanoma MALME-3M) by 30%. Further testing of **7** (100 μM to 10 nM) indicated that no LD_{50} could be found for any cell line at concentrations used in the antiangiogenic assay (Table S4, Supporting Information). Only five high LD_{50} values (25−92 μM) could be found for NCI-H460, M14, SK-MEL-5, IGROV1, and CAKI-1 cell lines. All cell lines showed some growth inhibition in the low μM concentration range (1−10 μM).

Protein Kinase Inhibition. Three compounds (ageladine A, **7**, and **9**) were screened against 402 protein kinases by Ambit Biosciences (Table S2, Supporting Information), showing the percentage of ATP binding remaining after treatment with the

dx.doi.org/10.1021/jm200039m | J. Med. Chem. 2011, 54, 2492–2503

Table 3. Anti-angiogenic Activity of Ageladine A and Selected Derivatives[a]

R=	compound	% inhibition at 100 μg/mL	% inhibition at 10 μg/mL)	% inhibition at 1 μg/mL	% inhibition at 0.1 μg/mL	IC_{50} μg/mL (R^2)
	**1**	100	19	10	9	24 (0.9121)
	**10**	41	14	–		150 (0.9615)
	**3**	37	–	–		–
	**4**	65	5	+2		59 (0.9781)
	**11**	99	29	23		17 (0.9536)
	**6**	57	14	–		75 (0.9982)
	**9**	77	2	6		49 (0.8904)
	**7**	100	56	16	4	8 (0.9761)
	**8**	3	–	–	–	–
	**14**	98	19	–	–	19 (0.9288)
	**15**	88	3	–	–	21 (0.9036)
	**16**	60	23	–	–	63 (0.9680)

[a] Control (just DMSO) was used as a standard to calculate the percentage inhibition. **13**, IC_{50} = 100 μg/mL was the positive control.

test compound. Values less than 50 suggest a K_D in the nM range. Ageladine A was tested at 10 μM and 1 μM and found to be very selective, with only DYRK1A, DYRK2, TYK2 (JH2 domain), and YSK4 showing activity of 5% or less at 10 μM (Table S2, Supporting Information). Compound **7** inhibited the same four kinases, but compound **9** had a different profile except that it also inhibited DYRK1A and TYK2.

From this primary screen, 19 kinases were selected for quantitative dissociation constant determination against **7** and **9**, using 11 concentrations and a blank (in duplicate). These data (Table 4 and Supporting Information) confirmed that a high nanomolar dissociation constant of **7** against DYRK1A, YSK4, and also RPS6KA1(domain 2). Analogue **9** also had a high nM K_D against DYRK1A, TYK2, PIK4CB, and PIK3C2G.

To further investigate the possible inhibitory activity of ageladine A, and its analogues, against protein kinases, we screened our compounds against a small panel of kinases in a functional assay using natural or synthetic substrates and

dx.doi.org/10.1021/jm200039m |J. Med. Chem. 2011, 54, 2492–2503

Table 4. Dissociation Constants (K_D) for Compounds 7 and 9 for Selected Kinases

	compound	
kinase target (Ambit gene symbol)	**7** K_D (nM)	**9** K_D (nM)
AAK1	1500	
BMPR1B		720
CAMK2G	40000[a]	
CAMKK1	40000	
CAMKK2	40000	
CDK7	7500	
CDKL5		40000
CLK2		1200
DYRK1A	290	300
DYRK2		970
IRAK3		8900
PIK3C2G		160
PIK4CB		350
PRKCE	40000	
RPS6KA1(Kin.Dom.2-C-terminal)	530	
TYK2 (JH2domain-pseudokinase)		290
ULK1	24000	
YANK2		40000
YSK4	220	

[a] 40000 indicates no binding observed.

radioactive ATP to measure any reduction in the rate of phosphorylation (Table 5). **1** was found to have moderate activity against DYRK1A and Pim 1 and weak activity against CK1 (not in the panel of 402 kinases tested by Ambit). Interestingly, the pyridine analogue (**7**) showed improved activity against CK1, which may be related to its ability to bind metal ions (Figure 1). Furthermore, while **1** has no activity against CDK5 and CDK1, most of the analogues did, with the pyridine analogue (**7**) again showing the best activity. The quinoline derivative (**9**) showed selective activity against DYRK1A.

DISCUSSION

Synthesis. Of the reported syntheses, the biomimetic approach has proved the most efficient.[6,13] Our aim was now to shorten the process to a one-pot reaction and expand our methodology to the synthesis of a number of analogues, which replace the pyrrole with other heterocycles, and thus investigate the role of the dibromopyrrole moiety in the biological activity of ageladine A.

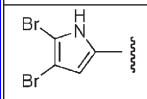
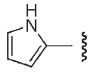
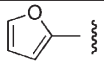
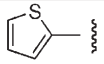
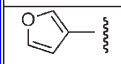
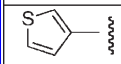
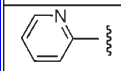
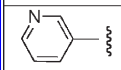
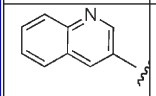
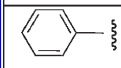
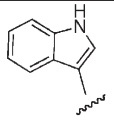
The yields obtained for the tetrahydro intermediates were excellent except for those that contained pyrrole/indole, in which case, yellow side products were obtained, reducing the yield. Boc protection did not help but did improve the solubility of the product for the next step. Dehydrogenation with chloranil was achieved in reasonable to good yield for all the analogues except those containing a pyridine (or quinoline). Other oxidizing agents such as S_8/DMSO, permanganate, or DDQ were not successful with any of the analogues. Similarly, solvents other than chloroform also reduced the yield of the desired products. We also tried the two-step (IBX, MnO_2) method of Ando[8] on the 2-furyl and 2-thiophenyl analogues but could only obtain low yields of the dehydrogenated product.

Palladium on carbon (Pd/C) has been used for the dehydrogenation of tetrahydro-β-carbolines using ethanol as a solvent.[14] This method was found to give excellent yields for the pyridine analogues, where all other methods had failed. Similar results were obtained for the other analogues (Table 1). The advantage of Pd/C over chloranil for dehydrogenation is that the intermediates are far more soluble in ethanol than the halogenated solvents required for chloranil oxidation and so do not require Boc-protection and the workup is trivial. As the Pictet−Spengler reaction was found not to require a catalyst, and because both steps now require the same solvent, we investigated the possibility of a one-pot total synthesis of ageladine A. Disappointingly, the reaction proceeded with a very low yield for the natural product and any analogue that contained a pyrrole. Boc protection of the pyrrole did improve the yields. but the results were still disappointing. Adding triethylamine or $Sc(OTf)_3$ did not improve the yields. However, to our delight, all other analogues gave excellent yields of the desired product using this one-pot procedure (Table 1). For the pyrrole containing systems, the two-step procedure, employing Boc-protection and choranil dehydrogenation, was found to deliver the best results, suggesting that the pyrrole-N*H* interferes with the Pd dehydrogenation. The success of the one-pot method allowed us to quickly generate a range of analogues and opens the door to industrial production of these compounds and further analogues.

Matrix Metalloprotease Inhibition. MMPs are a small family of zinc and calcium dependent proteases that are implicated in many disease states. The production of excess MMPs leads to the degradation of the extracellular matrix in connective tissue, which leads to disease symptoms including the loss of cartilage in osteoarthritis[15] and rheumatoid arthritis,[16] bone matrix degradation in osteoporosis, remodeling in Alzheimer's disease,[17] and cancer cell invasion and metastasis.[18] As the degradation of the extracellular matrix is critical for malignant tumor growth, invasion, metastasis, and angiogenesis,[19] MMP inhibition has become an emerging target for the treatment of cancers. To date, there are 27 known MMPs in humans and animals. On the basis of function and substrate specificity, they are classified as collagenases (MMP-1, -8, and -13), gelatinases (MMP-2 and -9), stromelysins (MMP-3, -10, and -11), membrane type (MT) MMPs (MT1 to MT6, MMP-14 to -17, -24, and -25), matrilysin (MMP-7), macrophage metalloelastases (MMP-12 and -19), and enamelysin (MMP-20).[20,21]

Ageladine A has shown inhibitory activity against MMP-1, -2, -8, -9, -12, and -13, suggesting they might be useful in cancer research and therapy.[9] The previous reports on the synthesis of analogues and their biological screening against MMPs suggested the importance of a free guanidine nucleus, pyrrole NH, and the presence of bromine atoms on the biological function of **1**.[1,4,8] Collectively, these studies indicated that removal of one or both bromines from ageladine A, or bromo-regioisomers, resulted in loss of activity against MMPs. Similarly, methylation of one or more nitrogens reduced or completely eliminated activity. The authors suggested that the pyrrole NH, two bromines and guanidine are essential for MMP inhibition. Our studies do not support this conclusion as, for example, the thiophene analogue (**4**) showed comparable activity to ageladine A. However, moving the sulfur atom (**6**) resulted in an order of magnitude drop in activity, suggesting that a heteroatom in the 2-position is important for activity.

Table 5. Effect of Ageladine A and Analogues on the Activity of Five Protein Kinases[a]

R=	Compound	CDK1/ cyclin B	CDK5/ p25	CK1	Dyrk1A	Pim 1	GSK3
	**1**	–	–	50	30	10	–
	**2**	–	70	–	–	–	–
	**3**	–	34	–	–	–	–
	**4**	–	43	–	80	–	–
	**5**	–	–	–	–	–	–
	**6**	40	11	–	11	–	–
	**7**	9	6	10	5	–	–
	**8**	–	60	–	10	–	–
	**9**	–	–	–	7	40	–
	**10**	–	40	–	40	–	–
	**12**	–	–	–	40	50	–

[a] Compounds were tested at various concentrations up to 100 μM and IC_{50} values (μM) were calculated from the dose−response curves. −, inactive at 100 μM

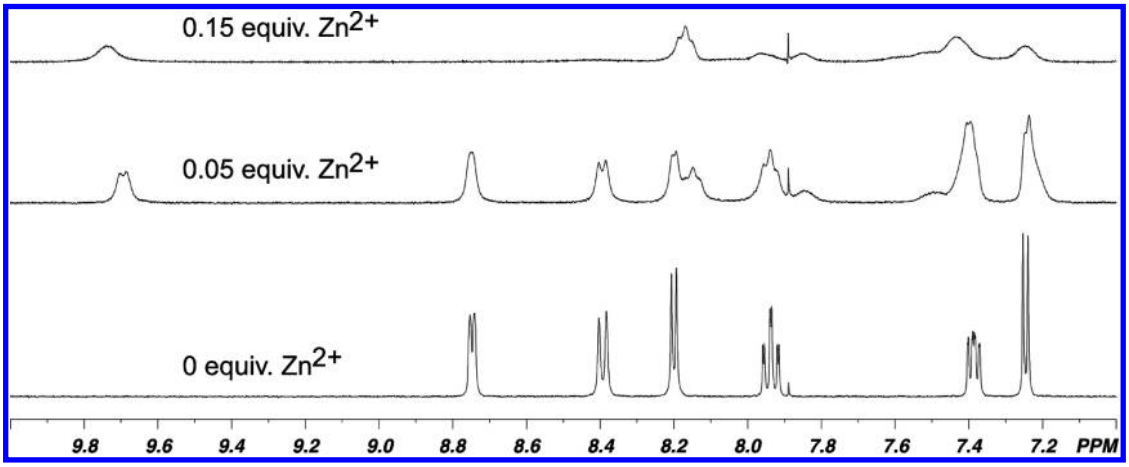


Figure 1. Titration of the free base of the 2-pyridyl analogue (**7**) with $ZnCl_2$ in methanol-d_4.

Our results indicate that the presence of pyrrole *NH* and bromine atoms is not absolutely necessary for MMP inhibitory activity and that removal of bromines (ie **1** going to **2**) results in loss of activity that could be due to a change in lipophilicity or steric bulk.

dx.doi.org/10.1021/jm200039m |*J. Med. Chem.* 2011, 54, 2492–2503

Most of the MMP inhibitors reported so far inhibit this family of enzyme by chelation of the zinc(II) ion at the proteases active site and are thus of restricted medicinal utility. Ageladine A is of interest because it has been shown to inhibit MMPs without binding zinc.[1] In contrast, **7** strongly complexes zinc, as determined by NMR spectroscopy (Figure 1). Adding 0.05 equiv of zinc chloride to a solution of **7** in CD_3OD resulted in splitting of the aromatic signals into two groups, consistent with the formation of a organometallic complex. Further addition of zinc led to considerable broadening of all signals. As **7** contains a 2,2′-dipyridyl moiety, this is not a totally unexpected result but interesting because this compound showed little inhibition of either MMP tested (Table 2), confirming that ageladine A and its analogues do not inhibit MMPs through zinc complexation.

Angiogenesis Inhibition. Angiogenesis plays a critical role in cancer growth and metastasis by providing tumors with an adequate blood supply.[22,23] An attractive recent strategy for new anticancer drugs is to target angiogenesis rather than directly eliminate tumor cells with highly cytotoxic agents.[22] The antiangiogenic activity of ageladine A was originally demonstrated by experiments carried out in vitro in a vascular organization model using mouse ES cells.[1]

At 10 μg/mL, ageladine A showed inhibition of vascular formation from aggregates of vascular progenitor cells in 3D culture using a type-I collagen gel, but its activity was not quantified nor compared to any known antiangiogenic drug. In this study, advantage was taken of a novel in vitro angiogenesis assay[10,24] to quantify the antiangiogenic activity of ageladine A and eight related compounds (Table 3). The assay involves culturing rat aorta vessels in a fibrin gel and measuring the effect of different substances on the growth of microvessels from the severed ends of the vessel fragment into the surrounding fibrin gel. This assay has been successfully used to screen for antiangiogenic compound in the past and was used to discover the antiangiogenic sulfated oligosaccharide, **13**,[11] which has undergone extensive clinical trials in cancer patients,[25] and was used as a positive control. Using this assay, we have quantified the antiangiogenic activity of ageladine A (IC_{50}) and 11 analogues and compared these to the known drug **13** (Table 3, Table S1, Supporting Information). The most striking feature is that there seems to be no correlation between MMP inhibition and inhibition of angiogenesis. For example, **11** showed virtually no MMP inhibition but antiangiogenic activity better than the natural product. Similarly, **4** showed the best MMP inhibition of the analogues yet showed only weak inhibition of angiogenesis. This complete lack of correlation suggests that the antiangiogenic activity of ageladine A is not closely related to its MMP inhibition or, at least, that MMP inhibition is not the natural product's primary mode of activity. Compounds **14**−**16** also showed that the guanidine, previously reported to be required for MMP inhibition, is not essential for antiangiogenic activity.

Cytotoxicity Screening. The observed antiangiogenic activity could be related to general cytotoxicity resulting in reduced vascular outgrowths in the angiogenesis assay due to cell death. To test this, ageladine A and **7** were tested against a panel of 60 human cancer cell lines. Little if any cytotoxicity was observed, but some growth inhibition was apparent for most cell lines at low μM concentration (Figure S2, Supporting Information). The five-dose assay over 48 h (Table S4, Supporting Information) for **7** differentiated growth inhibition (retardation of growth but no cell death) from cytotoxicity (retardation of growth due to cell death) and confirmed that **7** was noncytotoxic at <100 μM against all cell lines. These data indicated that while these compounds might inhibit growth of cancer cell lines they are not cytotoxic.

Kinase Screening. Protein kinases play an important role in the regulation of numerous cell functions including cell division cycle, gene expression, exocytosis, neuronal functions, membrane transport, and cellular differentiation. The human genome encodes 518 protein kinases comprising 90 tyrosine kinases, 338 serine/threonine kinases, and 50 sequences lacking a functional catalytic site.[26−29] Deregulation of kinase activities is frequently observed during the development of human diseases such as cancer, diabetes, Alzheimer's disease, inflammation, etc. This has stimulated an intensive search for low molecular weight pharmacological inhibitors (drugs) for protein kinases.[30−33] As the strong antiangiogenic activity of ageladine A cannot be fully explained by moderate MMP inhibition, we postulated that, as yet unidentified, protein kinases might constitute additional targets. In addition, the structure of ageladine A is reminiscent of hymenialdisine, a marine natural product that is a potent inhibitor of CDKs, Mek1, GSK3β, CK1, and Chk1 kinases.[34,35] An initial screen of 402 kinases showed moderate but highly selective activity. The assay, developed by Ambit Biosciences (San Diego, CA), uses phage-displayed natural and mutant kinases to assess K_D values against immobilized ATP.[36] The assay has been extensively validated and relies on the ability for a small molecule to bind to the ATP binding site of the tested kinases.[37] The selectivity scores (Table S3, Supporting Information) of greater than 35% inhibition at 1 μM for ageladine A (0.008), **7** (0.022), and **9** (0.008) are better than the most selective kinase drug (lapatinib S = 0.010).[37] Interestingly, the natural product inhibited mainly Ser/Thr kinases (70% of the top 10) and **7** inhibited almost exclusively these kinases (90% of top 10), while **9** inhibited mostly Tyr or inositol kinases (60% of top 10). Functional screening against a small panel of disease related kinases (Table 5) showed moderate activity against a small number of kinases, particularly DYRK1A.

These data, taken together, show moderate inhibition of selective kinases with DYRK1A being a common thread. Ageladine A and its antiangiogenic analogues may be inhibitors of DYRK1A or a related kinase. DYRK1A is a member of the dual-specificity tyrosine phosphorylation-regulated kinase family that contains a nuclear targeting signal sequence, a protein kinase domain, a leucine zipper motif, and a highly conservative 13-consecutive-histidine repeat. It catalyzes its own autophosphorylation on serine/threonine and tyrosine residues. It may play a significant role in a signaling pathway regulating cell proliferation and has recently been found to be involved in reduced tumor angiogenesis in Down's Syndrome.[38]

■ CONCLUSION

We have successfully developed an industrially useful, one-pot method for the synthesis of ageladine A and analogues using a biomimetic Pictet−Spengler cyclization between 2-aminohistamine/histamine/histadine and various heterocyclic aldehydes. The method is concise, robust, relatively cheap, and fast compared to previously published methods and can be used to make a diverse range of analogues by simply changing the starting materials. The robustness of the methodology has been demonstrated by the successful synthesis of 14 ageladine A analogues that differ only in the pendant heterocycle and the substitution pattern on the imidazole and pyridine rings. These analogues

have been tested for their MMP inhibitory effect. While none are as active as the natural product, several have comparable activity that has allowed a preliminary structure−activity relationship to be developed that can be used to synthesize second generation analogues. We have also shown, for the first time, that ageladine A also has kinase inhibitory activity and that some of the analogues are more active than the parent natural product in angiogenesis and kinase assays. These results suggest that ageladine A may target a kinase rather than an MMP to achieve its reported antiangiogenic activity. Testing of the most active kinase-inhibitory analogues showed that the 2-pyridine analogues (**7**, **14**, **15**) were highly active as antiangiogenic compounds. Surprisingly, the regioisomer 3-pyridine analogue (**8**) showed no kinase nor angiogenic activity. These data suggest that ageladine A may constitute a new kinase-inhibitory scaffold from which more potent and selective inhibitors might be designed.

GENERAL METHODS

Chloroform, dichloromethane, ethanol, and methanol were obtained from Froline Australia and were distilled before use. Merck Silica Gel 60 (230−400 mesh) was used for flash chromatography. All the reagents were obtained from commercial sources and used without further purification. NMR spectra were recorded in 5 mm Pyrex tubes (Wilmad, USA) on either a DPX-400 400 MHz or DPX-600 600 MHz spectrometer (Bruker, Germany). IR spectra were recorded on a Paragon PE1000 FTIR spectrophotometer (Perkin-Elmer, USA). Chemical shifts of ^{1}H NMR and ^{13}C NMR were referenced to the solvents peaks: δ_H 3.30 and δ_C 49.0 for CD_3OD and δ_H 2.49 and δ_C 39.2 for DMSO-d_6. A Phenomenex reverse phase HPLC column (Gemini, 5 μm, 150 mm × 2 mm) was used for LC-MS (LCMS-2010 EV equipped with a photodiode array detector of electrospray source) using water/acetonitrile/0.1% formic acid as mobile phase. All test compounds were purified to >95% purity by reverse phase HPLC using a 600E multisolvent delivery system and a 490 programmable multiwavelength detector (Waters, USA), using a Phenomenex Gemini column (10 μ, 250 mm × 10 mm) using a gradient from 5% acetonitrile/0.05% TFA to 80% acetonitrile/0.05% TFA. Purity was confirmed by ^{1}H and ^{13}C NMR spectroscopy (pp S20−S44, Supporting Information). All structures of all final compounds and intermediates were confirmed by ^{13}C and/or ^{1}H NMR spectroscopy and high-resolution mass spectrometry, measured on Bruker Apex 4.7T FTICR-MS. Ageladine A and all the analogues were characterized and stored as their TFA salts after HPLC.

General Procedure for Pictet−Spengler Cyclization of 2-Aminohistamine. a. To a stirred solution of 2-AH (0.11 mmol) or Boc-2AH (0.11 mmol) in ethanol (5 mL) was added triethylamine (0.16 mmol) and aldehyde (0.13 mmol). The reaction mixture was stirred at rt for 3−6 h, after which the solvent was removed under reduced pressure and the reaction mixture was further subjected to flash chromatography over silica gel using a gradient of 5:95−30:70 (MeOH:DCM saturated with ammonia) yielding the tetrahydro-intermediates (56−92%).

General Procedures for the Dehydrogenation of Tetrahydropyridine Intermediate. b. To a stirred solution of the tetrahydro-intermediate (0.15 mmol) in chloroform (10 mL) was added chloranil (0.30 mmol) and the reaction mixture refluxed for 15−24 h. Solvent was removed on a rotary evaporator and the residue obtained further purified by flash chromatography over silica gel with a gradient of 5:95−15:85 (MeOH:DCM saturated with ammonia), giving ageladine A analogues (Table 1).

c. To a stirred solution of tetrahydro-intermediate (0.15 mmol) in ethanol was added 10% Pd/C (30 mg) and the reaction mixture refluxed for 18−24 h. The mixture was then filtered through a Celite pad and washed with methanol (3 × 25 mL) and toluene:methanol (50:50; 2 × 20 mL), as many of these compounds were found to have a high affinity for charcoal. The combined filtrates were concentrated under reduced pressure and subject to flash chromatography over silica gel using a gradient of 5:95−20:80 (MeOH:DCM saturated with ammonia), yielding the ageladine A analogues (Table 1), which were further purified by HPLC as their TFA salts.

General Procedures for the One-Pot Synthesis of Ageladine A and Analogues. d. A mixture of 2-aminohistamine (0.11 mmol) and aldehyde (Table 1; 0.13 mmol) was stirred in ethanol at room temperature for 6 h, after which 10% Pd/C (24 mg) was added and the reaction mixture refluxed for a further 24 h. The mixture was then filtered through a Celite pad and washed with methanol (3 × 25 mL) and toluene:methanol (50:50; 2 × 20 mL). The combined filtrates were concentrated under reduced pressure and chromatographed over silica gel using a gradient of 5:95−20:80 (MeOH:DCM saturated with ammonia), giving **1**−**12** (Table 1) respectively.

e. To a stirred solution of histamine or histidine (0.180 mmol) in ethanol (2 mL) and aldehyde (0.180 mmol) was added potassium carbonate (2.16 mmol) and molecular sieves (3 Å; 200 mg). The reaction mixture was refluxed for 24 h, after which 10% Pd/C (60 mg) and cyclopentene (120 μL) were added. The reaction mixture was allowed to reflux for 2 days. The mixture was then filtered through a pad of Celite and washed with methanol (3 × 25 mL) and toluene:methanol (50:50; 2 × 20 mL). The combined filtrates were concentrated under reduced pressure and chromatographed over silica using a gradient of 2:98−15:85 (methanol:chloroform saturated with ammonia) to yield **14**−**16**.

Zinc Titration. The free base of analogue **7** was generated from the HPLC purified TFA salt by filter chromatography on silica gel (methanol/dichloromethane/ammonia). The free base (4.9 mg, 22 μmol) was then dissolved in CD_3OD (300 μL), followed by the addition of 0, 10, and 30 μL of $ZnCl_2$ (0.11 M in CD_3OD). ^{1}H NMR spectra were recorded on a Bruker DPX400 400 MHz NMR spectrometer.

MMP Inhibition Assays. Recombinant human MMP-2[39] and MT1−MMP[40] were prepared following the literature procedures. The fluorescent substrate; MOCAc-Pro-Leu-Gly-Leu-A_2pr(Dnp)-Ala-Arg-NH_2 was purchased from Peptide Institute Inc., Osaka. Inhibition assays were carried out by a modified procedure of Knight et al.[41] Test samples (2 μL), including positive (Batimostat, BB-94; IC_{50} 0.29, 1.45 μg/mL for MMP-2 and MT1-MMP respectively)[42] and negative (1% DMSO) controls, were added to wells of 96-well microtiter plates, each of which contained 100 μL of TNC buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 10 mM $CaCl_2$, 0.02% NaN_3 as a preservative and 0.05% Brij-35 detergent). Aliquots (50 μL) of enzyme solution (5 ng/mL for MMP-2 and 40 ng/mL for MT1-MMP) were added to this solution and preincubated at 37 °C for 10 min. After preincubation, 50 μL of substrate solution (10 μM) was added to the mixture to begin the reaction. Fluorescence values were measured at an excitation of 328 nm and an emission of 393 nm after incubation at 37 °C for 3 h. As all the ageladine A analogues are also fluorescent at 390 nm, a reference sample with all components except the enzyme was used as a blank and subtracted from all assay readings.

Angiogenesis Assay. An in vitro angiogenesis assay was used in this study to assess the antiangiogenic activity of the different compounds. The procedure, which has been described in detail previously,[24] used rat aorta fragments and is based on a procedure previously described by Brown et al.[10] using human placenta vessel fragments. Briefly, thoracic aortas were excised from 3−9 month-old female Fischer rats, cross-sectioned at 1 mm intervals and embedded individually in 0.5 mL of a fibrin gel containing 5 μg/mL of aprotinin (to prevent fibrinolysis) in each well of a 48-well culture plate. Immediately after vessel embedding, 0.5 mL/well of medium M199, supplemented with 20% fetal calf serum, 0.1% ε-aminocaproic acid, 1% L-glutamine,, and antibiotics, was added. Each test compound (10 mg/mL, dissolved in

DMSO) was added to the medium (maximum final concentration of test compound 100 μg/mL), and each treatment was performed in six replicates. Control cultures received medium containing 0.1% DMSO but without any test compound. The antiangiogenic compound, **13** (100 μg/mL), was included as a positive control. Vessels were cultured at 37 °C in 5% CO_2 in air for 5 days and the medium, with or without test compound, was changed on day 4. Vessel growth was quantified manually under 40× magnification on day 5, with growth being estimated as the percentage of the field (×40) around the vessel fragment that was occupied by vessel outgrowths. Inhibition of 100% indicates that no vessel outgrowth was observed, and 0 indicates the same amount as control, allowing the assay to be used for angiogenic as well as antiangiogenic compounds. The IC_{50} values were calculated by fitting the data to a standard Hill plot, using the blank as the zero value (nominally 0.01 μg/mL) and constraining the top value to be 100 (GraphPad Prism version 4.0).

Cytoxicity Screening. Was carried out by the NCI (Cancer Drug Discovery and Development Program). The screening is a two-stage process, beginning with the evaluation of all compounds against the 60 cell lines at a single dose of 10 μM. Compounds which exhibit significant growth inhibition are evaluated against the 60 cell panel at five concentration levels where LD_{50} and GI_{50} are quantified.[43]

Kinase Screening. Was conducted at Ambit Biosciences, San Diego, CA.[36] For most assays, kinase-tagged T7 phage strains were grown in parallel in 24-well blocks in an *Escherichia coli* host derived from the BL21 strain. *E. coli* were grown to log-phase and infected with T7 phage from a frozen stock (multiplicity of infection = 0.4) and incubated with shaking at 32 °C until lysis (90−150 min). The lysates were centrifuged (6000*g*) and filtered (0.2 μm) to remove cell debris. The remaining kinases were produced in HEK-293 cells and subsequently tagged with DNA for qPCR detection. Streptavidin-coated magnetic beads were treated with biotinylated small molecule ligands for 30 min at room temperature to generate affinity resins for kinase assays. The liganded beads were blocked with excess biotin and washed with blocking buffer (SeaBlock (Pierce), 1% BSA 0.05% Tween 20, 1 mM DTT), to remove unbound ligand and to reduce nonspecific phage binding. Binding reactions were assembled by combining kinases, liganded affinity beads, and test compounds in 1× binding buffer (20% SeaBlock, 0.17× PBS, 0.05% Tween 20, 6 mM DTT). Test compounds were prepared as 40× stocks in 100% DMSO and directly diluted into the assay. All reactions were performed in polypropylene 384-well plates in a final volume of 0.04 mL. The assay plates were incubated at room temperature with shaking for 1 h, and the affinity beads were washed with wash buffer (1× PBS, 0.05% Tween 20). The beads were then resuspended in elution buffer (1× PBS, 0.05% Tween 20, 0.5 mM nonbiotinylated affinity ligand) and incubated at room temperature with shaking for 30 min. The kinase concentration in the eluates was measured by qPCR.

Kinase Assay. Buffer A: 10 mM $MgCl_2$, 1 mM EGTA, 1 mM DTT, 25 mM Tris-HCl pH 7.5, 50 μg heparin/mL. Buffer C: 60 mM β-glycerophosphate, 15 mM *p*-nitrophenylphosphate, 25 mM Mops (pH 7.2), 5 mM EGTA, 15 mM $MgCl_2$, 1 mM DTT, 1 mM sodium vanadate, 1 mM phenyl phosphate.

Kinase activities were assayed in buffer A or C, at 30 °C, at a final ATP concentration of 15 μM. Blank values were subtracted and activities expressed as percent of the maximal activity, i.e. in the absence of inhibitors. Controls were performed with appropriate dilutions of DMSO, which was used to dissolve all test compounds.

CDK1/cyclin B (native M phase starfish oocytes) and *CDK5/p25* (recombinant human) were prepared as previously described.[35] Kinase activity was assayed in buffer C, with 1 mg histone H1/mL, in the presence of 15 μM [γ-^{32}P] ATP (3000 Ci/mmol, 10 mCi/mL) in a final volume of 30 μL. After 30 min incubation at 30 °C, 25 μL aliquots of supernatant were spotted onto 2.5 cm × 3 cm pieces of Whatman P81 phosphocellulose paper and, 20 s later, the filters were washed five times (for at least 5 min each time) in a solution of 10 mL of phosphoric acid/L of water. The wet filters were counted in the presence of 1 mL ACS (Amersham) scintillation fluid.

CK1 (porcine brain, native) was assayed as described for CDK1 but using the CK1-specific peptide substrate RRKHAAIGpSAYSITA,[44] obtained from Millegen (Labege, France).

DYRK1A (rat, recombinant, expressed in *E. coli* as a GST fusion protein) was purified by affinity chromatography on glutathione-agarose and assayed as described for *CDK1*/cyclin B using myelin basic protein (1 mg/mL) as a substrate.

Pim1 (human recombinant) was assayed with histone H1 as described for CDK1 and CDK5.

***N′-tert*-Butoxycarbonyl-5-(2-amino-4,5,6,7-tetrahydroimidazo[4,5-*c*]pyridine-4-yl)-2,3-dibromopyrrole.** To a stirred solution of 2-aminohistamine (10 mg, 79 μmol) in ethanol (5 mL) was added *N*-Boc-4,5-dibromo-2-formylpyrrole (27.9 mg, 79 μmol) and scandium triflate (3.8 mg, 7.9 μmol), and the reaction mixture stirred at room temperature for 5 h. The solvent was then removed in vacuo leaving a brown residue, which was purified by column chromatography on silica gel using a gradient of 5:95−15:85 (MeOH:DCM saturated with ammonia) to give tetrahydroageladine A as a yellow solid (16 mg, 44%). UV (acetonitrile/water) λ_{max} 220 nm, ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.61 (bs, 1H), 10.18 (bs, 1H), 5.97 (bs, 0.5H), 5.78 (bs, 0.5H), 5.52 (bs, 0.5H), 5.06 (bs, 2H), 4.11 (bs, 0.5H), 3.97 (bs, 1H), 3.06 (m, 1H), 2.5 (m, 1H), 2.33 (bm, 1H), 1.42 (s, 9H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 153.8, 149.2, 134.1, 125.0, 120.8, 109.6, 98.6, 96.5, 79.2, 49.1, 48.3, 39.1, 28.1 (3C), 22.0; *m/z* 360 (48%), 362 (100) 364 (52). HRMS found [M + Na^+] 483.9777, $C_{15}H_{19}N_5O_2{}^{79}Br^{81}BrNa$ requires 483.9777.

Ageladine A (1). To a stirred solution of tetrahydroageladine A (10 mg, 21.6 μmol) in chloroform (5 mL) was added chloranil (15.9 mg, 65 μmol), and the reaction mixture was heated at 80 °C (reflux) for 8 h. The solvent was removed in vacuo and the yellow residue subjected to column chromatography over silica using a gradient of 5:95−15:85 (MeOH:DCM saturated with ammonia) to yield pure ageladine A (**1**) as a yellow solid (5 mg, 65%). Ageladine A was characterized as its TFA salt (HPLC gradient 1:9−9:1 accetonitrile/water/0.1% TFA) for comparison to the original natural product. UV (acetonitrile/water) λ_{max} 210, 220 (sh), 248, 275 (sh), 290, 363, 380 (sh) nm. ^{1}H NMR (400 MHz, MeOD) δ 8.06 (d, *J* = 6.4 Hz, 1H), 7.42 (d, *J* = 6.4 Hz, 1H), 7.17 (s, 1H). ^{13}C NMR (100 MHz, MeOD) δ 160.8, 147.2, 136.7, 133.0, 128.5, 125.6, 115.1, 107.8, 105.4, 102.3; *m/z* 356 (53%), 358 (100) 360 (48).

***N′-tert*-Butyl-2-(2-amino-4,5,6,7-tetrahydro-1*H*-imidazo-[4,5-*c*]pyridine-4-yl)1*H*-pyrrole-1-carboxylate.** ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.15 (dd, *J* = 3.3, 1.8 Hz, 1H), 5.99 (t, *J* = 3.3 Hz, 1H), 5.82 (dd, *J* = 2.6, 1.8 Hz, 1H), 5.3 (s, 1H), 4.76 (bs, 2H), 3.01−2.80 (m, 2H), 2.54−2.28 (m, 2H), 1.58 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.6, 148.1, 135.3, 125.8, 124.2, 121.9, 115.2, 109.9, 84.1, 49.0, 38.8, 28.0 (3C), 23.2. IR (KBr) 3345, 2978, 1736, 1622, 1478, 1331, 1158, 1119, 1061, 845, 729 cm^{-1}. HRMS found [M + H]$^+$ 304.1781, $C_{15}H_{21}N_5O_2$ requires 304.1774.

4-(Pyrrole-2-yl)-1*H*-imidazo[4,5-*c*]pyridin-2-amine (2). ^{1}H NMR (400 MHz, CD_3OD) δ 7.93 (d, *J* = 6.5 Hz, 1H), 7.34 (d, *J* = 6.5 Hz, 1H), 7.23 (dd, *J* = 2.6, 1.4 Hz, 1H), 7.18 (dd, *J* = 4.0, 1.4 Hz, 1H), 6.42 (dd, *J* = 3.9, 2.6 Hz, 1H). ^{13}C NMR (100 MHz, CD_3OD) δ 160.2, 146.1, 136.4, 131.6, 131.5, 125.5, 123.4, 112.5, 111.8, 104.2. HRMS found [M + H]$^+$ 200.0932, $C_{10}H_9N_5$ requires 200.0936.

***N′′-tert*-Butyl-4-(furan-2-yl)-4,5,6,7-tetrahydro-1*H*-imidazo-[4,5-*c*]pyridine-2-ylcarbamate.** ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 (dd, *J* = 1.7, 0.8 Hz, 1H), 6.26 (dd, *J* = 3.1, 1.8 Hz, 1H), 5.89−5.87 (m, 1H), 5.22 (s, 1H), 2.97 (ddd, *J* = 13.0, 6.0, 1.7 Hz, 1H), 2.84 (ddd, *J* = 13.0, 10.7, 4.6 Hz, 1H), 2.60−2.49 (m, 1H), 2.34 (ddd, *J* = 16.2, 4.5, 1.2 Hz, 1H), 2.24 (bs, 1H), 2.23 (bs, 1H), 1.29 (s, 9H). ^{13}C NMR (100

MHz, MeOD) δ 155.3, 150.8, 149.9, 141.4, 134.8, 116.2, 110.2, 108.0, 85.2, 50.3, 38.3, 27.6 (3C), 25.5. IR (KBr) 3443, 1724, 1647, 1389, 1350, 1145, 1110, 837, 729 cm^{-1}. HRMS found $[M + H]^+$ 305.1612, $C_{15}H_{20}N_4O_3$ requires 305.1614.

4-(Furan-2-yl)-1*H*-imidazo[4,5-*c*]pyridin-2-amine (3). ^1H NMR (400 MHz, CD_3OD) δ 8.10 (d, *J* = 6.4 Hz, 1H), 7.92 (dd, *J* = 1.8, 0.7 Hz, 1H), 7.67 (dd, *J* = 3.6, 0.6 Hz, 1H), 7.44 (d, *J* = 6.4 Hz, 1H), 6.80 (dd, *J* = 3.5, 1.8 Hz, 1H). ^{13}C NMR (100 MHz, CD_3OD) δ 161.8, 150.3, 147.5, 145.4, 133.9, 133.4, 127.1, 116.9, 114.3, 107.1. HRMS found $[M + H]^+$ 201.0777, $C_{10}H_8N_4O$ requires 201.0776.

***N′′*-*tert*-Butyl-4-(thiophen-2-yl)-4,5,6,7-tetrahydro-1*H*-imidazo[4,5-*c*]pyridine-2-ylcarbamate.** ^1H NMR (400 MHz, $CDCl_3$) δ 7.20 (dd, *J* = 5.0, 1.2 Hz, 1H), 6.89 (dd, *J* = 5.0, 3.5 Hz, 1H), 6.70−6.68(m, 1H), 5.80 (bs, 1H), 5.40 (s, 1H), 3.00 (ddd, *J* = 13.3, 6.2, 2.1 Hz, 1H), 2.91 (ddd, *J* = 13.3, 10.2, 4.8 Hz, 1H), 2.56 (dddd, *J* = 16.4, 10.1, 6.3, 1.9 Hz, 1H), 2.30 (dddd, *J* = 16.4, 4.8, 2.1, 0.6 Hz, 1H), 2.23 (bs, 1H), 1.29 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 150.6, 149.9, 147.6, 134.5, 126.2, 125.7, 124.5, 118.5, 85.4, 51.8, 38.4, 27.5 (3C), 25.6. IR (KBr) 3470, 2929, 1714, 1647, 1388, 1346, 1146, 1102, 838, 809, 729 cm^{-1}. HRMS found $[M + H]^+$ 321.1393, $C_{15}H_{20}N_4O_2S$ requires 321.1385.

4-(Thiophen-2-yl)-1*H*-imidazo[4,5-*c*]pyridin-2-amine (4). ^1H NMR (400 MHz, CD_3OD) δ 8.10 (d, *J* = 6.4 Hz, 1H), 8.09 (dd, *J* = 3.8, 1.1 Hz, 1H), 7.88 (dd, *J* = 5.0, 1.1 Hz, 1H), 7.48 (d, *J* = 6.4 Hz, 1H), 7.32 (dd, *J* = 5.0, 3.8 Hz, 1H). ^{13}C NMR (100 MHz, CD_3OD) δ 161.3, 148.7, 136.9, 133.7, 133.0, 132.5, 132.2, 131.1, 129.3, 106.4. HRMS found $[M + H]^+$ 217.0539, $C_{10}H_8N_4S$ requires 217.0548.

4-(Furan-3-yl)-1*H*-imidazo[4,5-*c*]pyridin-2-amine (5). ^1H NMR (400 MHz, CD_3OD) δ 8.74 (m, 1H), 8.10 (d, *J* = 6.4 Hz, 1H), 7.81 (m, 1H), 7.49 (d, *J* = 6.4 Hz, 1H), 7.23 (m, 1H). ^{13}C NMR (100 MHz, CD_3OD) δ 161.4, 148.5, 147.5, 146.2, 137.8, 132.8, 131.2, 118.0, 108.7, 106.4; *m*/*z* 201 (100; M + H^+). HRMS found $[M + H]^+$ 201.0771, $C_{10}H_8N_4O$ requires 201.0776.

***N′′*-*tert*-Butyl-4-(thiophen-3-yl)-4,5,6,7-tetrahydro-1*H*-imidazo[4,5-*c*]pyridine-2-ylcarbamate.** ^1H NMR (400 MHz, $CDCl_3$) δ 7.27−7.24 (m, 1H), 7.02 (dd, *J* = 4.9, 1.3 Hz, 1H), 6.82−6.80 (m, 1H), 5.80 (bs, 1H), 5.23 (s, 1H), 2.99−2.92 (m, 1H), 2.86−2.78 (m, 1H), 2.60−2.50 (m, 1H), 2.41−2.33 (m, 1H), 2.20 (bs, 1H), 1.24 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 150.5, 150, 144.6, 134.2, 127.7, 125.4, 122.4, 118.8, 85.1, 52.0, 38.5, 27.4, (3C) 25.8. IR (KBr) 3447, 3106, 1724, 1647, 1388, 1346, 1259, 1142, 1107, 839, 748 cm^{-1}. HRMS found $[M + H]^+$ 321.1397, $C_{15}H_{20}N_4O_2S$ requires 321.1385.

4-(Thiophen-3-yl)-1*H*-imidazo[4,5-*c*]pyridine-2-amine (6). ^1H NMR (400 MHz, CD_3OD) δ 8.60 (dd, *J* = 2.9, 1.3 Hz, 1H), 8.11 (d, *J* = 6.4 Hz, 1H), 7.90 (dd, *J* = 5.2, 1.3 Hz, 1H), 7.73 (dd, *J* = 5.1, 2.9 Hz, 1H), 7.51 (d, *J* = 6.4 Hz, 1H). ^{13}C NMR (100 MHz, CD_3OD) δ 161.9, 150.3, 137.0, 133.2, 132.7, 131.9, 130.8, 128.9, 127.2, 107.0. HRMS found $[M + H]^+$ 217.0545, $C_{10}H_8N_4S$ requires 217.0548.

4-(Pyridine-2-yl)-4,5,6,7-tetrahydro-1*H*-imidazo[4,5-*c*]pyridine-2-amine. ^1H NMR 400 MHz, CD_3OD) δ 8.51 (ddd, *J* = 4.9, 1.7, 0.9 Hz, 1H), 7.79−7.73 (m, 1H), 7.36−7.26 (m, 2H), 4.92 (t, *J* = 1.8 Hz, 1H), 3.14−3.07 (m, 1H), 3.01−2.93 (m, 1H), 2.65−2.50 (m, 2H). ^{13}C NMR (100 MHz, CD_3OD) δ 162.1, 150.7, 150.1, 138.4, 126.5, 124.9, 124.3, 124.0, 59.0, 42.0, 24.0. IR (KBr) 3404, 1620, 1573, 1473, 1435, 1092, 752 cm^{-1}. HRMS found $[M + H]^+$ 216.1246, $C_{11}H_{13}N_5$ requires 216.1249.

4-(Pyridine-2-yl)-1*H*-imidazo[4,5-*c*]pyridin-2-amine (7). ^1H NMR (400 MHz, CD_3OD) δ 8.89−8.80 (m, 2H), 8.28 (d, *J* = 6.2 Hz, 1H), 8.07−8.02 (m, 1H), 7.57−7.52 (m, 2H). ^{13}C NMR (100 MHz, CD_3OD) δ 161.6, 160.5, 150.8, 150.0, 148.9, 139.0, 135.9, 133.8, 126.5, 125.0, 107.7. HRMS found $[M + H]^+$ 212.0932, $C_{11}H_9N_5$ requires 212.0936.

4-(Pyridine-3-yl)-4,5,6,7-tetrahydro-1*H*-imidazo[4,5-*c*]pyridine-2-amine. ^1H NMR 400 MHz, CD_3OD) δ 8.49−8.46 (m, 1H), 8.43 (dd, *J* = 4.9, 1.6 Hz, 1H), 7.75−7.71 (m, 1H), 7.38 (ddd, *J* = 7.8, 4.9, 0.8 Hz, 1H), 4.89 (t, *J* = 1.7 Hz, 1H), 3.12−3.04 (m, 1H), 3.01−2.93 (m, 1H), 2.68−2.59 (m, 1H), 2.57−2.48 (m, 1H). ^{13}C NMR (100 MHz, CD_3OD) δ 150.8, 150.5, 149.1, 139.9, 138.4, 127.1, 125, 124.7, 55.9, 42.2, 23.8. IR (KBr) 3317, 1620, 1574, 1475, 1425, 1094, 1028, 801, 713 cm^{-1}. HRMS found $[M + H]^+$ 216.1241, $C_{11}H_{13}N_5$ requires 216.1249.

4-(Pyridine-3-yl)-1*H*-imidazo[4,5-*c*]pyridin-2-amine (8). ^1H NMR (400 MHz, CD_3OD) δ 9.28 (d, *J* = 1.9 Hz, 1H), 8.79 (dd, *J* = 5.0, 1.5 Hz, 1H), 8.59−8.55 (m, 1H), 8.30 (d, *J* = 6.6 Hz, 1H), 7.76 (ddd, *J* = 8.0, 5.0, 0.8 Hz, 1H), 7.60 (d, *J* = 6.6 Hz, 1H). ^{13}C NMR (100 MHz, CD_3OD) δ 162.0, 151.5, 150.0, 149.7, 138.8, 137.6, 135.4, 133.8, 129.1, 125.7, 107.9. HRMS found $[M + H]^+$ 212.0928, $C_{11}H_9N_5$ requires 212.0936.

4-(Quinoline-3-yl)-4,5,6,7-tetrahydro-1*H*-imidazo[4,5-*c*]-pyridine-2-amine. ^1H NMR 400 MHz, CD_3OD) δ 8.82 (d, *J* = 2.1 Hz, 1H), 8.17 (d, *J* = 2.1 Hz, 1H), 8.01 (dd, *J* = 8.5, 0.9 Hz, 1H), 7.89 (dd, *J* = 8.1, 1.4 Hz, 1H), 7.75−7.70 (m, 1H), 7.60−7.55 (m, 1H), 5.00 (t, *J* = 1.8 Hz, 1H), 3.16−3.08 (m, 1H), 3.05−2.98 (m, 1H), 2.73−2.64 (m, 1H), 2.61−2.52 (m, 1H). ^{13}C NMR (100 MHz, CD_3OD) δ 152.4, 150.7, 148.2, 137.5, 136.5, 130.9, 129.4, 129.3, 128.9, 128.2, 126.8, 124.6, 55.9, 42.2, 23.8. IR (KBr) 3422, 2925, 1620, 1573, 1473, 1124, 861, 752 cm^{-1}. HRMS found $[M + H]^+$ 266.1406, $C_{15}H_{15}N_5$ requires 266.1406.

4-(Quinoline-3-yl)-1*H*-imidazo[4,5-*c*]pyridin-2-amine (9). ^1H NMR (400 MHz, CD_3OD) δ 9.53 (d, *J* = 2.2 Hz, 1H), 9.11 (d, *J* = 2.2 Hz, 1H), 8.33 (d, *J* = 6.4 Hz, 1H), 8.18−8.12 (m, 2H), 7.98−7.92 (m, 1H), 7.80−7.75 (m, 1H), 7.62 (d, *J* = 6.3 Hz, 1H). ^{13}C NMR (100 MHz, CD_3OD) δ 161.9, 149.7, 148.3, 139.7, 137.7, 135.8 (br), 135.5, 133.7 (br), 133.5, 130.2, 129.5, 128.8, 128.7, 125.5, 107.9. HRMS found $[M + H]^+$ 262.1091, $C_{15}H_{11}N_5$ requires 262.1093.

4-Phenyl-1*H*-imidazo[4,5-*c*]pyridin-2-amine (10). ^1H NMR (400 MHz, CD_3OD) δ 8.13 (d, *J* = 5.4 Hz, 1H), 7.90 (d, *J* = 7.4 Hz, 2H), 7.54−7.48 (m, 2H), 7.46−7.41 (m, 1H), 7.19 (d, *J* = 5.5 Hz, 1H). ^{13}C NMR (100 MHz, CD_3OD) δ 148.8, 136.3, 134.9, 131.9, 129.7, 129.4, 128.9, 120.0, 117.1, 114.2, 111.4, 107.5; *m*/*z* 211 (100; M + H^+). HRMS found $(M + H)^+$ 211.0987, $C_{12}H_{10}N_4$ requires 211.0984.

***N′′*-*tert*-Butyl-4-(benzo[*b*]thiophen-3-yl)-4,5,6,7-tetrahydro-1*H*-imidazo[4,5-*c*]pyridine-2-ylcarbamate.** ^1H NMR (400 MHz, $CDCl_3$) δ 7.94 (d, *J* = 7.7 Hz, 1H), 7.85−7.81 (m, 1H), 7.42−7.29 (m, 2H), 6.83 (s, 1H), 5.86 (bs, 1H), 5.57 (s, 1H), 2.96 (ddd, *J* = 13.1, 5.9, 2.1 Hz, 1H), 2.82 (ddd, *J* = 13.1, 10.3, 4.7 Hz, 1H), 2.63−2.53 (m, 1H), 2.43−2.35 (m, 1H), 2.17 (bs, 1H), 1.02 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 150.8, 150.0, 140.9, 137.9, 134.9, 124.4, 124.2, 124.1, 123.0, 122.2, 117.8, 85.1, 50.8, 38.6, 27.4 (3C), 25.8. IR (KBr) 3453, 2928, 1722, 1627, 1369, 1326, 1263, 1138, 835, 765, 734 cm^{-1}. HRMS found $[M + H]^+$ 371.1547, $C_{19}H_{22}N_4O_2S$ requires 371.1542.

4-(Benzo[*b*]thiophen-3-yl)-1*H*-imidazo[4,5-*c*]pyridin-2-amine (11). ^1H NMR (400 MHz, CD_3OD) δ 8.29 (s, 1H), 8.27 (d, *J* = 6.5 Hz, 1H), 8.10−8.07 (m, 1H), 7.79−7.75 (m, 1H), 7.60 (d, *J* = 6.5 Hz, 1H), 7.55−7.48 (m, 2H). ^{13}C NMR (100 MHz, CD_3OD) δ 159.2, 158.8, 151.7, 141.7, 137.8, 135.8, 133.3, 126.9, 126.6, 124.2, 123.5, 117.4, 114.6, 109.0. HRMS found $[M + H]^+$ 267.0706, $C_{14}H_{10}N_4S$ requires 267.0704.

***N′′*-*tert*-Butyl-3-(2-amino-4,5,6,7-tetrahydro-1*H*-imidazo-[4,5-*c*]pyridine-4-yl)1*H*-indole-1-carboxylate.** ^1H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, *J* = 8.3 Hz, 1H), 7.42 (d, *J* = 7.7 Hz, 1H), 7.36 (s, 1H), 7.24−7.19 (m, 1H), 7.13−7.08 (m, 1H), 4.99 (s, 1H), 3.11−3.00 (m, 1H), 2.93−2.82 (m, 1H), 2.50−2.27 (m, 2H), 1.59 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.7, 148.1, 135.8, 129.4, 124.5, 124.4, 122.6, 121.9, 119.7, 115.2, 83.7, 49.9, 49.0, 41.6, 28.2 (3C), 23.7. IR (KBr) 3334, 2977, 1735, 1635, 1562, 1453, 1371, 1308, 1255, 1154,

1091, 855, 747 cm^{-1}. HRMS found $[M + H]^+$ 354.1940, $C_{19}H_{23}N_5O_2$ requires 354.1930.

4-(1*H*-Indole-3-yl)-1*H*-imidazo[4,5-*c*]pyridine-2-amine (12). ^1H NMR (400 MHz, CD_3OD) δ 8.15 (s, 1H), 8.12 (d, J = 6.59 Hz, 1H), 7.87−7.83 (m, 1H), 7.79−7.55 (m, 1H), 7.46 (d, J = 6.6 Hz, 1H), 7.46 (d, J = 6.6 Hz, 1H), 7.34−7.23 (m, 2H). ^{13}C NMR (100 MHz, CD_3OD) δ 162.0, 138.4, 134.3, 133.5, 130.2, 125.7, 124.4, 122.5, 120.1, 113.4, 107.3, 106.1. HRMS found $[M + H]^+$ 250.1087, $C_{14}H_{11}N_5$ requires 250.1093.

4-(Pyridin-2-yl)-3*H*-imidazo[4,5-*c*]pyridine (14). ^1H NMR (400 MHz, CD_3OD) δ 9.52 (d, J = 7.2 Hz, 1H), 8.98 (bs, 1H), 8.91 (s, 1H), 8.61 (d, J = 5.9 Hz, 1H), 8.23 (t, J = 7.7 Hz, 1 H), 8.19 (d, J = 5.9 Hz, 1H), 7.75 (bs, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 150.1, 148.9, 147.4, 146.5, 140.0, 138.6, 135.9, 134.7, 127.0, 126.5, 111.99. HRMS found $[M + H]^+$ 197.0824, $C_{11}H_9N_4$ requires 197.0822.

4-(Pyridin-2-yl)-3*H*-imidazo[4,5-*c*]pyridine-6-carboxylic Acid (15). Obtained as the free base after column chromatography as colorless crystals (48%). ^1H NMR (400 MHz, DMSO-d_6) δ 8.91−8.89 (m, 2H), 8.76 (s, 1H), 8.48 (s, 1H), 8.20 (dt, J = 7.8, 1.6 Hz, 1H), 7.67 (dd, J = 7.0, 5.3 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.1, 154.8, 149.4, 149.3, 148.7, 140.8, 140.7, 139.5, 125.7, 123.1, 116.7. HRMS found $[M + H]^+$ 241.0714, $C_{12}H_9N_4O_2$ requires 241.0720.

4-(6-Bromopyridin-2-yl)-3*H*-imidazo[4,5-*c*]pyridine-6-carboxylic Acid (16). Obtained as a pale-yellow solid after HPLC (71%). ^1H NMR (400 MHz, DMSO-d_6) δ 12.68 (bs, 1H), 8.76 (d, J = 7.72 Hz, 1H), 8.68 (s, 1H), 8.48 (s, 1H), 8.05 (t, J = 7.8 Hz, 1H), 7.83 (dd, J = 7.8, 0.78 Hz, 1H). ^{13}C NMR (150 MHz, DMSO-d_6 at −10 °C) δ 168.7, 158.3, 152.3, 149.6, 142.7, 141.8, 140.6, 132.4, 130.5, 122.9, 119.7. LRMS (ESI) m/z 319 (100%), 321 (98%). HRMS found $[M + H]^+$ 318.9828, $C_{12}H_8N_4O_2{}^{79}Br$ requires 318.9825

■ ASSOCIATED CONTENT

Supporting Information. Raw data for the screening of a subset of compounds against 402 human kinases and calculation of IC_{50} values, selectivity values, and treespot diagrams. Calculations for antiangiogenic activity and cytoxicity data against 60 human cell lines. All ^1H and ^{13}C spectra for synthetic products. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +612 9850 8290. Fax: +612 9850 8313. E-mail: peter.karuso@mq.edu.au.

■ ACKNOWLEDGMENT

We thank Macquarie University for an iMURS scholarship (SRS). The authors are indebted to Professor Motoharu Seiki (University of Tokyo) for providing the MMP enzymes used in this study and Anna Bezos for performing the angiogenesis assays. This research was supported in part, by grants from the EEC (FP6-2002-Life Sciences and Health, PRO-KINASE Research Project) (L.M., J.E.), the Canceropole Grand-Ouest grant (L.M.), the Association France-Alzheimer Finistere (L.M.), and the Ligue Nationale contre le Cancer (L.M.). CRP is supported by a NHMRC Program grant.

■ ABBREVIATIONS USED

2-AH, 2-aminohistamine; MMP, matrix metalloprotease; MT1-MMP, membrane type 1 metalloprotease; IBX, 2-iodoxybenzoic acid; DCM, dichloromethane; DDQ, 2,3-dichloro-5,6-dicyanobenzoquinone; TNC, tris/sodium chloride/calcium chloride buffer; MOCAc−, 7-methoxycoumarin-4-acetyl−; NCI, National Cancer Institute; ES cells, embryonic stem cells

■ REFERENCES

(1) Fujita, M.; Nakao, Y.; Matsunaga, S.; Seiki, M.; Itoh, Y.; Yamashita, J.; van Soest, R. W. M.; Fusetani, N. Ageladine A: an antiangiogenic matrixmetalloproteinase inhibitor from the marine sponge *Agelas nakamurai*. *J. Am. Chem. Soc.* **2003**, *125*, 15700–15701.

(2) Nakao, Y.; Fusetani, N. Enzyme inhibitors from marine invertebrates. *J. Nat. Prod.* **2007**, *70*, 689–710.

(3) Meketa, M. L.; Weinreb, S. M. Total synthesis of ageladine A, an angiogenesis inhibitor from the marine sponge *Agelas nakamurai*. *Org. Lett.* **2006**, *8*, 1443–1446.

(4) Meketa, M. L.; Weinreb, S. M.; Nakao, Y.; Fusetani, N. Application of a 6π-1-azatriene electrocyclization strategy to the total synthesis of the marine sponge metabolite ageladine A and biological evaluation of synthetic analogues. *J. Org. Chem.* **2007**, *72*, 4892–4899.

(5) Andrade, P.; Willoughby, R.; Pomponi, S. A.; Kerr, R. G. Biosynthetic studies of the alkaloid, stevensine, in a cell culture of the marine sponge *Teichaxinella morchella*. *Tetrahedron Lett.* **1999**, *40*, 4775–4778.

(6) Shengule, S. R.; Karuso, P. Concise total synthesis of the marine natural product ageladine A. *Org. Lett.* **2006**, *8*, 4083–4084.

(7) Meketa, M. L.; Weinreb, S. M. A new total synthesis of the zinc matrixmetalloproteinase inhibitor ageladine a featuring a biogenetically patterned 6 π-2-azatriene electrocyclization. *Org. Lett.* **2007**, *9*, 853–855.

(8) Ando, N.; Terashima, S. Synthesis and matrix metalloproteinase (MMP)-12 inhibitory activity of ageladine A and its analogs. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4495–4499.

(9) Mannello, F.; Tonti, G.; Papa, S. Matrix metalloproteinase inhibitors as anticancer therapeutics. *Curr. Cancer Drug Targets* **2005**, *5*, 285–298.

(10) Brown, K. J.; Maynes, S. F.; Bezos, A.; Maguire, D. J.; Ford, M. D.; Parish, C. R. A novel in vitro assay for human angiogenesis. *Lab. Invest.* **1996**, *75*, 539–555.

(11) Parish, C. R.; Freeman, C.; Brown, K. J.; Francis, D. J.; Cowden, W. B. Identification of sulfated oligosaccharide-based inhibitors of tumor growth and metastasis using novel in vitro assays for angiogenesis and heparanase activity. *Cancer Res.* **1999**, *59*, 3433–3441.

(12) Shoemaker, R. H. The NCI60 human tumour cell line anticancer drug screen. *Nature Rev. Cancer* **2006**, *6*, 813–823.

(13) Ando, N.; Terashima, S. Synthesis and matrix metalloproteinase (MMP)-12 inhibitory activity of ageladine A and its analogs. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4495–4499.

(14) Mahboobi, S.; Wiegrebe, W.; Popp, A. Synthesis of enantiomerically pure (−)-(*S*)-brevicolline. *J. Nat. Prod.* **1999**, *62*, 577–579.

(15) Lohmander, L. S.; Hoerrner, L. A.; Lark, M. W. Metalloproteinases, tissue inhibitor, and proteoglycan fragments in knee synovial fluid in human osteoarthritis. *Arthritis Rheum.* **1993**, *36*, 181–189.

(16) Murphy, G.; Hembry, R. M. Proteinases in rheumatoid arthritis. *J. Rheumatol.* **1992**, *19*, 61–64.

(17) Peress, N.; Perillo, E.; Zucker, S. Localization of tissue inhibitor of matrix metalloproteinases in Alzheimer's disease and normal brain. *J. Neuropathol. Exp. Neurol.* **1995**, *54*, 16–22.

(18) Chang, C.; Werb, Z. The many faces of metalloproteases: cell growth, invasion, angiogenesis and metastasis. *Trends Cell Biol.* **2001**, *11*, S37–S43.

(19) Tosetti, F.; Ferrari, N.; De Flora, S.; Albini, A. "Angioprevention": angiogenesis is a common and key target for cancer chemopreventive agents. *FASEB J.* **2002**, *16*, 2–14.

(20) Pendás, A. M.; Knäuper, V.; Puente, X. S.; Llano, E.; Mattei, M.-G.; Apte, S.; Murphy, G.; López-Otín, C. Identification and characterization of a novel human matrix metalloproteinase with unique structural characteristics, chromosomal location, and tissue distribution. *J. Biol. Chem.* **1997**, *272*, 4281–4286.

(21) Matter, H.; Schudok, M. Recent advances in the design of matrix metalloprotease inhibitors. *Curr. Opin. Drug Discovery Dev.* **2004**, *7*, 513–535.

(22) Folkman, J. Angiogenesis: an organizing principle for drug discovery? *Nature Rev. Drug Discovery* **2007**, *6*, 273–286.
(23) Folkman, J. Angiogenesis. *Annu. Rev. Med.* **2006**, *57*, 1–18.
(24) Sydnes, M. O.; Bezos, A.; Burns, C.; Kruszelnicki, I.; Parish, C. R.; Su, S.; Rae, A. D.; Willis, A. C.; Banwell, M. G. Synthesis and biological evaluation of some enantiomerically pure C8c-C15 monoseco analogues of the phenanthroquinolizidine-type alkaloids cryptopleurine and julandine. *Aust. J. Chem.* **2008**, *61*, 506–520.
(25) Khachigian, L. M.; Parish, C. R. Phosphomannopentaose sulfate (PI-88): heparan sulfate mimetic with clinical potential in multiple vascular pathologies. *Cardiovasc. Drug Rev.* **2004**, *22*, 1–6.
(26) Ohya, T. Identification of 4-methylspinaceamine, a Pictet–Spengler condensation reaction product of histamine with acetaldehyde, in fermentated foods and its metabolite in human urine. *J. Agric. Food. Chem.* **2006**, *54*, 6909–6915.
(27) Manning, G.; Whyte, D. B.; Martinez, R.; Hunter, T.; Sudarsanam, S. The protein kinase complement of the human genome. *Science* **2002**, *298*, 1912–1934.
(28) Kostich, M.; English, J.; Madison, V.; Gheyas, F.; Wang, L.; Qiu, P.; Greene, J.; Thomas, M. Human members of the eukaryotic protein kinase family. *Genome Biol.* **2002**, *3*, research0043.1–research0043.12.
(29) Neidner, R. H.; Buzko, O. V.; Hatse, N. M.; Taylor, A.; Gribskov, M.; Taylors, S. S. Protein kinase resource: an integrated environment for phosphorylation research. *Proteins* **2006**, *63*, 78–86.
(30) Fischer, P. M. The design of drug candidate molecules as selective inhibitors of therapeutically relevant protein kinases. *Curr. Med. Chem.* **2004**, *11*, 1563–1583.
(31) Weinmann, H.; Metternich, R. Drug discovery process for kinase inhibitors. *ChemBioChem* **2005**, *6*, 455–459.
(32) Smith, P. J.; Yue, E. *CDK Inhibitors of Cyclin-Dependent Kinases As Anti-tumor Agents*; Monographs on Enzyme Inhibitors; CRC Press, Taylor & Francis: Boca Raton, FL, 2006; Vol. 2, p 448.
(33) Meijer, L.; Flajolet, M.; Greengard, P. Pharmacological inhibitors of glycogen synthase kinase 3. *Trends Pharmacol. Sci.* **2004**, *25*, 471–480.
(34) Wan, Y. Q.; Hur, W. Y.; Cho, C. Y.; Liu, Y.; Adrian, F. J.; Lozach, O.; Bach, S.; Mayer, T.; Fabbro, D.; Meijer, L.; Gray, N. S. Synthesis and target identification of hymenialdisine analogs. *Chem. Biol.* **2004**, *11*, 247–259.
(35) Meijer, L.; Thunnissen, A. M. W. H.; White, A. W.; Garnier, M.; Nikolic, M.; Tsai, L. H.; Walter, J.; Cleverley, K. E.; Salinas, P. C.; Wu, Y. Z.; Biernat, J.; Mandelkow, E. M.; Kim, S. H.; Pettit, G. R. Inhibition of cyclin-dependent kinases, GSK-3β and CK1 by hymenialdisine, a marine sponge constituent. *Chem. Biol.* **2000**, *7*, 51–63.
(36) Fabian, M. A.; Biggs, W. H., III; Treiber, D. K.; Atteridge, C. E.; Azimioara, M. D.; Benedetti, M. G.; Carter, T. A.; Ciceri, P.; Edeen, P. T.; Floyd, M.; Ford, J. M.; Galvin, M.; Gerlach, J. L.; Grotzfeld, R. M.; Herrgard, S.; Insko, D. E.; Insko, M. A.; Lai, A. G.; Lelias, J. M.; Mehta, S. A.; Milanov, Z. V.; Velasco, A. M.; Wodicka, L. M.; Patel, H. K.; Zarrinkar, P. P.; Lockhart, D. J. A small molecule-kinase interaction map for clinical kinase inhibitors. *Nature Biotechnol.* **2005**, *23*, 329–336.
(37) Karaman, M. W.; Herrgard, S.; Treiber, D. K.; Gallant, P.; Atteridge, C. E.; Campbell, B. T.; Chan, K. W.; Ciceri, P.; Davis, M. I.; Edeen, P. T.; Faraoni, R.; Floyd, M.; Hunt, J. P.; Lockhart, D. J.; Milanov, Z. V.; Morrison, M. J.; Pallares, G.; Patel, H. K.; Pritchard, S.; Wodicka, L. M.; Zarrinkar, P. P. A quantitative analysis of kinase inhibitor selectivity. *Nature Biotechnol.* **2008**, *26*, 127–132.
(38) Baek, K.-H.; Zaslavsky, A.; Lynch, R. C.; Britt, C.; Okada, Y.; Siarey, R. J.; Lensch, M. W.; Park, I.-H.; Yoon, S. S.; Minami, T.; Korenberg, J. R.; Folkman, J.; Daley, G. Q.; Aird, W. C.; Galdzicki, Z.; Ryeom, S. Down's syndrome suppression of tumour growth and the role of the calcineurin inhibitor DSCR1. *Nature* **2009**, *459*, 1126–1130.
(39) Itoh, Y.; Takamura, A.; Ito, N.; Maru, Y.; Sato, H.; Suenaga, N.; Aoki, T.; Seiki, M. Homophilic complex formation of MT1-MMP facilitates proMMP-2 activation on the cell surface and promotes tumor cell invasion. *EMBO J.* **2001**, *20*, 4782–4793.
(40) Kinoshita, T.; Sato, H.; Takino, T.; Itoh, M.; Akizawa, T.; Seiki, M. Processing of a precursor of 72-kilodalton type IV collagenase/gelatinase A by a recombinant membrane-type 1 matrix metalloproteinase. *Cancer Res.* **1996**, *56*, 2535–2538.
(41) Knight, C. G.; Willenbrock, F.; Murphy, G. A novel coumarin-labeled peptide for sensitive continuous assays of the matrix metalloproteinases. *FEBS Lett.* **1992**, *296*, 263–266.
(42) Davies, B.; Brown, P. D.; East, N.; Crimmin, M. J.; Balkwill, F. R. A synthetic matrix metalloproteinase inhibitor decreases tumor burden and prolongs survival of mice bearing human ovarian carcinoma xenografts. *Cancer Res.* **1993**, *53*, 2087–2091.
(43) Alley, M. C.; Scudiero, D. A.; Monks, A.; Hursey, M. L.; Czerwinski, M. J.; Fine, D. L.; Abbott, B. J.; Mayo, J. G.; Shoemaker, R. H.; Boyd, M. R. Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. *Cancer Res.* **1988**, *48*, 589–601.
(44) Reinhardt, J.; Ferandin, Y.; Meijer, L. Purification of CK1 by affinity chromatography on immobilised axin. *Protein Expression Purif.* **2007**, *54*, 101–109.